Serial Number: 10/666,412 Filing Date: September 18, 2003

Title: Electroactive Microspheres and Methods

Dkt: 37000-UT-0206

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IN THE CLAIMS

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Please amend the claims as follows:

1. (Currently amended) A method of analyzing a sample for the presence of a member of a specific binding pair, the method comprising:

providing a <u>polymeric</u> microsphere having an electroactive <u>molecule</u> marker encapsulated within the <u>polymeric</u> microsphere and a first member of a specific binding pair attached to the <u>polymeric</u> microsphere wherein the <u>polymeric</u> microsphere is not a liposome;

introducing a sample suspected to comprise a second element of the specific binding pair complex to the <u>polymeric</u> miscrosphere;

selecting for the <u>polymeric</u> microsphere by formation of a specific binding pair complex in fluid suspension; and

releasing the electroactive molecule from the polymeric microsphere with an organic solvent; and

detecting the specific binding pair complex by electrochemical testing via voltammetry or amperometry for the electroactive molecule marker released from the polymeric microsphere.

wherein,

electrochemical testing is via voltammetry or amperometry.

- 2. (Currently amended) The method of claim 1 wherein the polymeric microsphere is a polymeric microsphere that is insoluble in an aqueous solution.
- 3. (Currently amended) The method of claim 2 wherein the <u>polymeric</u> microsphere is a polystyrene-based microsphere.
 - 4. (Cancelled)

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5. (Currently amended) The method of claim 1, wherein the providing step comprises incubationing of a the polymeric microsphere in an organic solvent including the electroactive markermolecule.

6. (Cancelled)

- 7. (Currently amended) The method of claim 1 wherein the selecting step comprises binding of the first member of the specific binding pair attached to the polymeric microsphere and a second member of the specific binding pair attached to a substrate.
- 8. (Currently amended) The method of claim 7 wherein the first member of the specific binding pair attached to the polymeric microsphere comprises a covalent bond with a functional group on the surface of the microsphere.
- 9. (Original) The method of claim 7 wherein the substrate comprises a magnetic particle.
- 10. (Currently amended) The method of claim 1 wherein the selecting step comprises incubationing.
- 11. (Currently amended) The method of claim 1 wherein the specific binding pair complex is comprises a pair selected from the group consisting of an antigen/antibody, enzyme/substrate, oligonucleotide/DNA, chelator/metal, enzyme/inhibitor, bacteria/receptor, virus/receptor, hormone/receptor, DNA/RNA, RNA/RNA, or-and an oligonucleotide/RNA complex.

12. (Cancelled)

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- 13. (Currently amended) The method of claim 1 wherein the releaseding step comprises solubilizing the polymeric microsphere.
- 14. (Currently amended) The method of claim 1 wherein the electroactive marker molecule comprises a metallocene.
- 15. (Currently amended) The method of claim 1 wherein the electroactive marker molecule comprises a nanoparticle.
- 16. (Currently amended) The method of claim 1 wherein the electroactive marker molecule comprises a metal.

17-19. (Cancelled)

20. (Currently amended) A method of analyzing a sample for the presence of two or more analytes, the method comprising:

providing a first <u>polymeric</u> microsphere having a first electroactive <u>markermolecule</u> incorporated into thea body of the first <u>polymeric</u> microsphere;

providing a second <u>polymeric</u> microsphere having a second electroactive markermolecule electrochemically distinguishable from the first electroactive markermolecule encapsulated within the body of the second <u>polymeric</u> microsphere wherein neither the first <u>polymeric</u> microsphere nor the second <u>polymeric</u> micropshere is a liposome;

attaching a first binding pair member specific to a first analyte to the first polymeric microsphere;

attaching a second binding pair member specific to a second analyte to the second polymeric microsphere;

incubating the first <u>polymeric</u> microsphere and second <u>polymeric</u> microsphere in a solution comprising the sample to be analyzed;

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selecting for the first polymeric microsphere and second polymeric microsphere by formation of specific binding pair complexes in fluid suspension; and

detecting the specific binding pair with electrochemical testingvia voltammetry or amperometry for the first electroactive markermolecule and the second electroactive markermolecule released from the first polymeric microsphere and the second polymeric microsphere.

wherein.

electrochemically detection is via voltammetry or amperometry

- 21. (Currently amended) The method of claim 20 wherein at least one of the first or second polymeric microspheres is a polymeric microsphere that is insoluble in an aqueous solution.
- 22. (Currently amended) The method of claim 21 wherein at least one of the first or second polymeric microspheres is a polystyrene-based microsphere.
- 23. (Currently amended) The method of claim 20 wherein the attaching step comprises forming a covalent bond with a functional group on thea surface of the polymeric microsphere.
- 24. (Currently amended) The method of claim 20 wherein the specific binding pair complexes are is selected from the group consisting of an antigen/antibody, enzyme/substrate, oligonucleotide/DNA, chelator/metal, enzyme/inhibitor, bacteria/receptor, virus/receptor, hormone/receptor, DNA/RNA, RNA/RNA, or and an oligonucleotide/RNA complexes.
- 25. (Currently amended) The method of claim 20 further comprising the step of releasing the first electroactive markermolecule from the first polymeric microsphere and the second electroactive markermolecule from the second polymeric microsphere.

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- 26. (Currently amended) The method of claim 2025 wherein the releasing step comprises solubilizing the first polymeric microsphere and the second polymeric microsphere.
- 27. (Currently amended) The method of claim 20 wherein the first electroactive markermolecule and the second electroactive markermolecule comprise metallocenes.
- 28. (Currently amended) The method of claim 20 wherein the first electroactive markermolecule and the second electroactive markermolecule comprise nanoparticles.
- 29. (Currently amended) The method of claim 20 wherein the first electroactive markermolecule and the second electroactive markermolecule comprise metal.

30-32. (Cancelled)

- 33. (Withdrawn) A microsphere for electrochemical detection of a member of a specific binding pair, comprising a polymeric microsphere having an organic solvent soluble hydrophobic electroactive marker incorporated into the body of the microsphere and at least one functional group on the surface of the microsphere.
- 34. (Withdrawn) The microsphere of claim 33 wherein the soluble hydrophobic electroactive marker is non-magnetic.
- 35. (Withdrawn) The microsphere of claim 34 wherein the soluble hydrophobic electroactive marker is a metallocene.
- 36. (Withdrawn) The microsphere of claim 35 wherein the metallocene is ferrocene or ferrocenecarboxaldehyde.

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- 37. (Withdrawn) The microsphere of claim 33 wherein the at least one functional group is a sulfate surface group, aldehyde group, aliphatic amine group, amide group, aromatic amine group, carboxylic acid group, chloromethyl group, epoxy group, hydrazide group, hydroxyl group, sulfonate group or tosyl group.
- 38. (Withdrawn) The microsphere of claim 33 wherein the polymeric microsphere is a polystyrene-based microsphere.
- 39. (Withdrawn) The microsphere of claim 38 wherein the polystyrene-based microsphere has a diameter between about 0.01 μm and about 100.0 μm .
- 40. (Withdrawn) The microsphere of claim 38 wherein the polystyrene-based microsphere has a diameter between about 0.3 μm and about 20 μm .